Biochemistry

BIOCHEMICAL CHARACTERIZATION OF THE DISEASE-CAUSING MUTATION IN DOMAIN SIX OF WILSON PROTEIN

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Abstract

Copper is an essential cofactor for many enzymes, including cytochrome oxidase, superoxide dismutase, and ceruloplasmin; as a result, a certain amount of copper is necessary for normal functioning in humans. On the other hand, excessive copper can be toxic. Wilson Disease is one of the most common causes of elevated copper content. It occurs because of mutations in the gene coding the Wilson Protein (ATP7B), a copper-transporting ATPase. One of these mutations is G591D in domain six of the protein. In order to characterize this mutation, domain six of Wilson Protein was overexpressed in BL21(DE3) cells. Domain six was purified by freeze/thaw extraction followed by two column chromatography steps: DEAE anion-exchange, and gel filtration. Following purification of the wild-type domain six, mutagenesis was performed to change the glycine at position 591 to aspartic acid. After expressing domain 6, it will then be possible to compare mutant and wild-type versions of domain six of Wilson Protein and characterize the mutation.